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# Role of vascular endothelial growth factor in the response to vessel injury

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**Role of vascular endothelial growth factor in the response to vessel injury.** In the post-embryonic life, physiological angiogenesis is tightly controlled. Angiogenesis also occurs in pathological circumstances such as tumor vessel proliferation, retinal neovascularization and ischemia. The development of collateral circulation is not only not deleterious, but life saving. Other cases such as neoplastic neovascularization are the basis of the continuous growth of tumors and metastases, and therefore constitute a target of therapeutical efforts. Among a list of molecules able to control angiogenesis, we emphasize the pivotal role of vascular endothelial growth factor (VEGF). VEGF is a potent mitogen for endothelial cells, but is devoid of mitogenic activity for other cell types. VEGF is a polypeptide with four main different isoforms that are remarkably different in terms of solubility and affinity for matrix proteins. VEGF interacts with two endothelial cell-specific tyrosine kinase receptors. The main interest of its study lies in VEGF's role in pathological angiogenic processes, where an increase in the VEGF mRNA expression has been consistently observed. An interesting example is the up-regulation of VEGF's and VEGF receptors' mRNA in a considerable number of human tumors and retina, where they have a critical role in the development of neovascularization. In recent work in our laboratory, we have found further potential interactions of VEGF with pathophysiological mechanisms, namely, the increase in VEGF gene expression under exposure to reactive oxygen species and the positive interaction between VEGF and erythropoietin. VEGF has outstanding possibilities for therapeutic applications aimed at inhibiting or favoring the development of new vessels.

The present article deals with aspects related to the role of vascular endothelial growth factor (VEGF) in angiogenesis, that is, in vascular proliferation from pre-existing vessels. This process is typical of the extrauterine life. Data on vasculogenesis, the generation of vessels from undifferentiated precursors that are typical of embryonic life, are not included. VEGF has important roles in both vasculogenesis and angiogenesis.

In the post-embryonic life, angiogenesis, or new microvessel growth, is tightly controlled and only works in conditions of vascular repair after injury or in strictly regulated mechanisms, for example, in skeletal or uterine muscular hypertrophy, ovarian cycle or placental development. The definition of "injury" broadly spans from the interruption of vessel continuity by mechanical means to the lack of nourishment in the vascular occlusion process and the damage by oxidant metabolites such as reactive oxygen species. Reactive oxygen species (ROS) denote compounds that are or that will generate oxygen free radicals.

Angiogenesis also occurs in pathological circumstances. Typical examples of angiogenesis occurring in pathological processes are tumor-associated vessel proliferation, retinal neovascularization in diabetes mellitus and the collateral neovascularization appearing in conditions of hypoxia, such as the action that occurs in ischemic organs. Some of these cases, for example, the development of collateral circulation, are not only not deletereous but life saving. Others such as neoplastic neovascularization are the basis of the continuous growth of tumors and metastases, and therefore constitute targets for therapeutical efforts.

There are substantial differences between the diverse angiogenic processes. First, in the endothelial growth in neoplasms, the process starts from the secretion by the tumor cells of factors with chemotactic and proliferative activity on endothelial cells (paracrine mode). Second, in hypoxia, the neovascularization mechanisms are not sufficiently clear, but the signal factors seem to be produced by the vascular tissues (autocrine mode). In both cases, the genetic program of endothelial cells triggers a phenotypic change to an angiogenic mode. This change implies the activation of a sequence that includes the production of proteolytic enzymes, migration, proliferation and, finally, a return to the previous differentiated state in the new vessels.

Work done by several researchers all over the world in the last few years has searched for the regulators of angiogenesis and has yielded numerous candidates. Table 1

**Key words:** vascular endothelial growth factor, KDR/flk-1 receptor, flt-1 receptor, vascular injury.

**Table 1.** Agents with proliferative effects on cells of the vascular wall

| Agent | EC | VSMC |
|---|---|---|
| PDGF | yes/not | yes |
| aFGF | yes | yes |
| bFGF | yes | yes |
| EGF | yes | yes |
| TGF-$\alpha$ | not | yes/not |
| ET-1 | yes | yes |
| VEGF | yes | not |

Abbreviations are: PDGF, platelet-derived growth factor; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; EGF, endothelial growth factor; TGF-$\alpha$, transforming growth factor-$\alpha$; ET-1, endothelin-1; EC; endothelial cells; VSMC, vascular smooth muscle cells.

**Table 2.** Cellular species which produce VEGF

| |
|---|
| Tumors |
| Follicular cells of the anterior pituitary |
| Vascular smooth muscle cells |
| Mesangial cells |
| Monocytes |
| Fibroblasts |
| Cheratinocytes |
| Osteoblasts |
| Astrocytes |

**Table 3.** Stimulatory mechanisms of VEGF expression and/or production

| |
|---|
| Hypoxia |
| AGEs |
| IL-1 |
| IL-6 |
| IGF-I |
| ROS |
| TGF-$\beta$ |
| Angiotensin II |
| Adenosine |
| Transitional metals |

Abbreviations are: AGEs, advanced glycosylation end products; IL-1, interleukin-1; IL-6, interleukin-6; IGF-1, insulin like growth factor I; EGF, epidermal growth factor; TGF, transforming growth factor; ROS, reactive oxygen species.

**Table 4.** Vascular endothelial growth factor: Biological effects

| |
|---|
| Endothelial cells mitogenesis |
| Increase in vascular permeability |
| Induction of the expression of the serine proteases |
| Induction of the expression of the interstitial collagenase |
| Induction of vasodilation and nitric oxide production |
| Stimulation of hexoses transport |

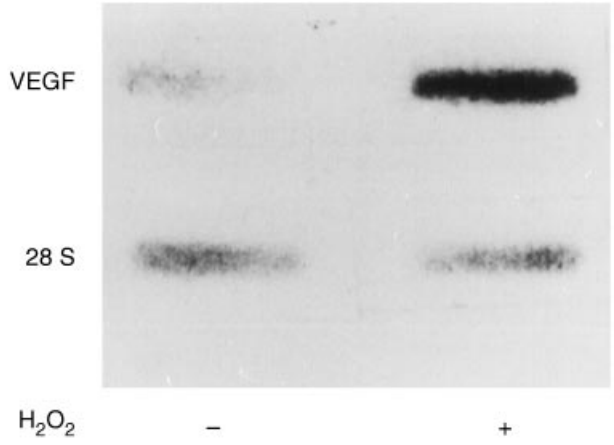


**Fig. 1. Increased expression of vascular endothelial growth factor (VEGF) in vascular smooth muscle cells (VSMC) treated with reactive oxygen species (ROS).**

shows a list of molecules that are able to control angiogenesis, which work as positive factors of endothelial and vascular smooth muscle cell (VSMC) proliferation. Among all of these agents, we want to highlight the pivotal role of VEGF. VEGF is a potent mitogen for micro- and macrovascular endothelial cells, but is devoid of appreciable mitogenic activity for other cell types, including those in the vascular wall, that is, vascular smooth muscle cells and pericytes. This fact has led to the hypothesis that VEGF may be a crucial function in the regulation of physiological and pathological growth of blood vessels.

VEGF is a polypeptide with a relative molecular mass of 45,OOO D. The alternative mRNA splicing of a single gene implies the existence of four different isoform species of 121, 165, 189 and 206 amino acids ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$). There are remarkable differences between these isoforms in terms of solubility and affinity for matrix proteins containing heparin and heparinoid residues. $VEGF_{121}$ has a weakly acid character and behaves as a soluble protein that fails to bind heparin. The predominant molecular species, $VEGF_{165}$, is also diffusible, although an important fraction remains bound to the extracellular matrix. The $VEGF_{189}$ and the $VEGF_{206}$ are more basic and bind heparin with greater affinity, and they are sequestered in the extracellular matrix. Transcripts encoding $VEGF_{121}$ and $VEGF_{189}$ are detected in the majority of cells and tissues expressing VEGF. In contrast, $VEGF_{206}$ production has only been identified in human and murine fetal liver cDNA libraries [1].

VEGF is secreted in a regulated fashion by several cellular types (Table 2). A group of mechanisms have been found to participate in the regulation of VEGF gene expression and/or to induce the release of VEGF protein (Table 3) [1].

VEGF is endowed with a series of biological effects. The most relevant among them are shown on Table 4. The actions of VEGF occur through interactions with its endothelial cell-specific tyrosine kinase receptors, i.e., the kinase domain region (human)/fetal liver kinase (murine) (KDR/flk-1) and the fms-like tyrosine kinase (flt-1). Both types of receptors have different signal transduction properties. KDR/flk-1 produces a strong ligand-dependent tyrosine kinase phosphorylation and generates a proliferative effect,

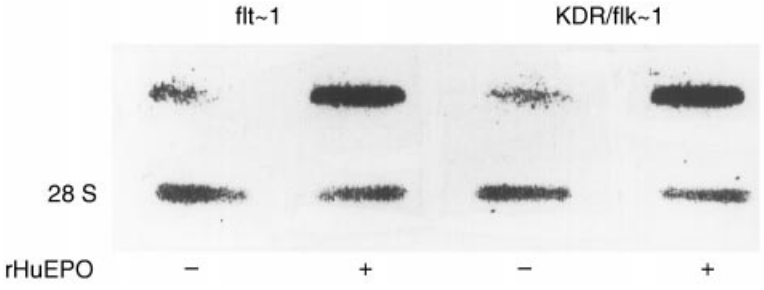


**Fig. 2. Stimulation of VEGF receptors KDR/flk-1 and flt-1 mRNA expression in endothelial cells treated with recombinant human erythropoietin (rHUEPO).**

while flt-1 has a weak VEGF-dependent tyrosine phosphorylation on endothelial cells and does not appear to generate mitogenic signals [1]. Even though total or partial expression of VEGF receptors has been recently described in other cellular types such as osteoblasts or mesangial cells, their role(s) is not yet defined.

The main interest in the study of VEGF lies in its role in pathological angiogenesis. In these pathological angiogenic processes, an increase in the VEGF mRNA expression has been observed, opening the possibility of relevant therapeutic applications. An interesting example is the upregulation of VEGF and VEGF receptors mRNA in a considerable number of human tumors. The available information indicates that VEGF has a critical role in the development of tumor-associated neovascularization [2–4]. Another important pathological condition involving VEGF is the neovascularization of diabetic proliferative retinopathy [5].

In recent work in our laboratory, we have found further potential interactions of VEGF with potential pathophysiological mechanisms. As an example, a marked increase in the VEGF gene expression and in the release of VEGF protein was observed in human vascular smooth muscle cells treated with reactive oxygen species (Fig. 1). Furthermore, the existence of a positive interaction between VEGF and the erythroid stimulating hormone, erythropoietin, was detected in endothelial cells treated with recombinant human erythropoietin. This interaction involved a higher proliferative response to VEGF and a potentiated increase in the VEGF-induced intracellular calcium transient. A possible explanation for these results is related to an additional finding, namely, the increased expression of the KDR/flk-1 and flt-1 receptor genes in endothelial cells treated with recombinant human erythropoietin (Fig. 2) [6].

The therapeutic applications of VEGF are basically related to the attempt of increasing or decreasing tissue VEGF concentration. This has been done by means of direct administration, gene transfer with cDNA encoding VEGF or inhibition by antisense oligonucleotides in cases of ocular neovascularization. These approaches included, for example, the development of collateral vessels in the hind limb ischemia and stent endothelization for revascularization procedures [7, 8]. It is rather predictable that these new approaches will reach a more extensive field of application in the near future.

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